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INSTITUTE FOR SPACE BIOSCIENCES

FLORIDA STATE UNIVERSITY TALLAHASSEE, FLORIDA

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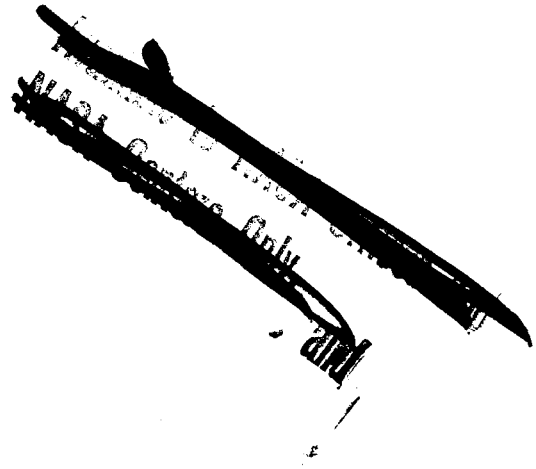
SECOND ANNUAL REPORT



1 November 1963

OUTLINE

RELATIONSHIP TO FIRST ANNUAL REPORT

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RELATIONSHIP TO FIRST ANNUAL REPORT

The history of the institute, the objectives, the working space, problems of outer space being investigated by others on the campus, list of antecedent publications, and an overall acknowledgment are set forth in the First Annual Report. They are not repeated in this Second Annual Report.

PERSONNEL

Name	Highest Degree	Title	Field of Investigation
C. R. Austin	Ph.D.	Visiting Professor	Reproductive Physiology
S. W. Fox	Ph.D.	Professor (and Director)	Molecular Evolution
S. Hess	Ph.D.	Professor	Comparative Meteorology
T. R. Mann	Ph.D.	Visiting Professor	Biochemistry
C. B. Metz	Ph.D.	Professor	Developmental Biology
L. Wiese	Ph.D.	Visiting Professor	Developmental Biology
K. Bahadur	Ph.D.	Visiting Research Associate	Molecular Evolution
D. Durant	Ph.D.	Visiting Research Associate	Molecular Evolution
K. Harada	Ph.D.	Research Associate	Molecular Evolution
T. Hayakawa	University Degree	Visiting Research Associate	Polymer Chemistry
G. Krampitz	Ph.D.	Visiting Research Associate	Molecular Evolution
C. L. Mann	Ph.D.	Visiting Investigator	Developmental Biology
M. Menzel	Ph.D.	Guest Investigator	Cytogenetics
C. A. Shivers	Ph.D.	Visiting Investigator (U.S.P.H.S. Post-doctoral Fellow)	Developmental Biology
P. Babcock	D.D.S.	Research Assistant	Developmental Biology
E. Bradley	M. S.	Research Assistant	Chemistry
R. Cheng	B. S.	Research Assistant	Meteorology
T. Fukushima	B. S.	Research Assistant	Developmental Biology
D. Hampson	B. S.	Research Assistant	Chemistry
D. Joseph	B. S.	Research Assistant	Molecular Evolution
R. Kendrick	B. S.	Research Assistant	Chemistry
R. McCauley		Research Assistant	Molecular Evolution
W. Wiese	M. S.	Research Assistant	Developmental Biology
E. Wiggert	M. S.	Research Assistant	Chemistry
C. Windsor	B. S.	Research Technician	Chemistry

Name	Highest Degree	Title	Field of Investigation
G. Brown	M. S.	Graduate Research Assistant	Developmental Biology
R. T. Brown	M. S.	Graduate Research Assistant	Meteorology
R. Castillo	M. S.	Graduate Research Assistant	Meteorology
L. Franklin	B. S.	Graduate Research Assistant	Developmental Biology
C. Genaux	M. S.	Graduate Research Assistant	Chemistry
A. Gruber	B. S.	Graduate Research Assistant	Meteorology
R. Hadlock	M. S.	Graduate Research Assistant	Meteorology
P. D. Hoagland	M. S.	Graduate Research Assistant	Chemistry
D. Rohlfing	M. S.	Graduate Student (U.S.P.H.S. Pre-doctoral Fellow)	Chemistry
A. Schwartz	M. S.	Graduate Research Assistant	Chemistry
S. Stern	M. S.	Graduate Research Assistant	Developmental Biology
K. Stewart	B. S.	Graduate Student (U.S.P.H.S. Pre-doctoral Fellow)	Chemistry
W. Adkins		Student Lab Assistant	
J. Bell		Student Lab Assistant	
J. Hasencamp		Student Lab Assistant	
C. McGahey		Student Lab Assistant	
W. Malthouse		Student Lab Assistant	
J. Murphy		Student Lab Assistant	
S. Steen		Student Lab Assistant	

Name	Title
N. Allen	Secretary
V. Bowser	Secretary
J. Carter	Administrative Assistant (half time)
J. Cox	Secretary (resigned)
M. E. Dockendorf	Executive Officer
M. Franklin	Executive Secretary (resigned)
J. Goldinger	Curator-Stockroom Assistant
H. Hendry	Expeditor and Accountant
J. Miller	Machinist's Assistant
L. Roddenberry	Administrative Assistant (half time)
G. Smith	Machinist's Assistant
N. Smith	Machinist
S. Sportelli	Stockroom Clerk
B. Whaley	Executive Secretary
R. Whitten	Stockroom Clerk
R. Williams, Jr.	Machinist's Assistant

PROGRESS IN RESEARCH

Wakulla Springs Conference on The Origins of Prebiological Systems (Fox, Dockendorf, et al.)

A conference on The Origins of Prebiological Systems was organized and held at the Wakulla Springs Lodge, Florida, on 27-30 October 1963. The participants from elsewhere were twenty four. Representatives from abroad were S. Akabori (Japan), J. D. Bernal (England, in absentia), J. B. S. Haldane (India), A. I. Oparin (U.S.S.R.), N. W. Pirie (England), and G. Schramm (Germany). This event had historical significance in that it was the first occasion on which Professors Oparin and Haldane met in person, although the earliest theory in this field is often referred to as the Oparin-Haldane theory.

Many aspects of the theory and experiments of abiogenesis were reviewed and many new results were reported.

The entire proceedings is being prepared for publication by the Academic Press under the title The Origins of Prebiological Systems and of Their Molecular Matrices, (S. W. Fox, Editor).

Extraterrestrial Macromolecular Sampler (Durrum, Fox, Wiggert, Windsor)

This study is being conducted collaboratively with Dr. Emmett Durrum of Durrum Instruments under a subgrant from NsG-173-62.

Of the many approaches which are possible the first is one of attempting to develop procedures for testing for polyamino acids. A preferred procedure would be one which will yield other information such as that bearing on the presence of pyrimidines. The first study concerned the possibility of separating on chromatograms hydrolyzates which would yield an amino acid profile after only four hours of hydrolysis. This was done with the restrictions of the Martian telemetering system in mind. The amino acid profiles were very much like that obtained after virtually complete hydrolysis such as results from 12-24 hours of processing, except that the peak in the area of histidine was found to be confused. The total recovery was roughly 2/3 of that obtained from complete hydrolysis, with no very great departure from this figure for any individual amino acid.

The next study was one of determining whether chromogenic reagents could withstand the temperatures indicated for sterilization. Surprisingly, ninhydrin was found not only not to lose any activity when heated at 145^{o} for 36 hours if heated dry; it actually gains activity slightly. This gain may be due to loss of water, yielding more activity per milligram. This result permits visualizing the use of ninhydrin in heat-sterilized vehicles. The possible behavior was suggested and tested because of the fact that amino acids are not easily destroyed by heat if they are heated dry.

Thermal Synthesis of Amino Acids (Harada, Fox)

In line with the developing thermal theory, "primordial" gases have been subjected to vapor phase reaction in beds of silica sand, silica gel, alumina gel, and lava sand. Results with the two kinds of silica are similar, the others different. On silica at 950-1050° C, fourteen amino acids are obtained from the reaction products of methane, ammonia, and water by acid hydrolysis. Two others which may be present, histidine and tryptophan, will have to be identified by special means. No cystine or methionine is formed; this is easily understood as due to the fact that the reaction mixture contained no sulfur compounds. The absence of cystine and methionine is part of the evidence that the amino acids do not arise by microbial contamination. No amino acids not occurring in protein are found in the reaction products from silica.

On alumina, only four proteinaceous amino acids, as by electric discharge, are found.

Organic Chemistry in Thermal Terrestrial Locales (Fox)

Temperatures and Dryness

The temperature range of 120-200° within inches of the surface of cinder cones at Kilaeua-Iki and Kapoho were found in extensive zones in July 1963, several years after the eruptions which formed these cones. The temperatures were read on two Weston bimetallic thermometers. At Kilaeua-Iki they were checked by Mr. Raymond J. Geerdes, ranger of the Hawaiian Volcanoes National Park. This information came as a surprise to several volcanologists stationed in the vicinity. Some of these experts developed and expressed interest in following temperature profiles of cinder cones in the future.

Wherever the temperature was above 100°, the samples taken were of course dry. These physical conditions are the necessary ones for the processes which have been demonstrated in the laboratory. Bullard (Volcanoes: In History, In Theory, In Eruption) has emphasized the widespread nature of large thermal

areas on the surface of the earth during its geological history.

Polymerization and Spherulization on Heated Lava (Fox, McCauley)

On samples of lava taken directly from Kapoho, the dry mixture of eighteen amino acids common to protein, with sufficient aspartic acid and glutamic acid, have been heated at 170° in the open air of the laboratory for 3 - 6 hours. Upon raining bacteria-free water on the hot reaction or washing with bacteria-free 1% saline, the polymer formed yields astronomical numbers of the usual microspheres. No units result without the amino acids on the lava.

The rugged and rapid nature of this sequence of natural processes under quasigeochemical conditions is thus reaffirmed.

Amino Acids from Volcanic Zones (Fox, Windsor)

Many of the samples have been analyzed. They are found to contain twelve to fourteen amino acids. Exclusion of the possibility of terrestrial contamination of the surfaces which were sampled is difficult, perhaps impossible. However, the amino acid profiles are similar, qualitatively and quantitatively, to those obtained by thermal synthesis. The possibility that these amino acids resulted from geosynthesis thus seems more likely.

A movie titled "Polymerization of Amino Acids and Formation of Microspheres on Heated Lava" has been made of this experiment and has a limited availability on request. Plans are being developed for public showings. The movie has proved to be unnecessary for experts with adequate chemical and geological orientation but has already been signally helpful in demonstrating to others the geological plausibility or probability of key thermal processes. Even for the well oriented expert, information such as the fact that unwashed lava can be directly used has proved to be helpful.

Origin and Retention of Optical Activity (Harada, Rohlfing, Fox)

Several new models of how optical activity might have originated were developed during the year. The number of these varied concepts, many published, from this laboratory is now so considerable as to merit comprehensive treatment in a subsequent annual report.

Fractionation of Proteinoids (Krampitz, Fox)

Dr. Krampitz has been able to fractionate proteinoid on CMC, DEAE, and Dowex-IX2 columns. Approximately 10 major fractions are obtained, containing over 100 minor fractions. The distribution of fractions is far from random. Some of the fractions are being analyzed.

Conversion of Cystine to Other Amino Acids (Genaux, Fox)

Since the thermal polymerization of amino acids has been studied in this laboratory, some amino acids have been found to be thermally copolymerizable (aspartic acid, arginine, glutamic acid, histidine, lysine) and others to be thermally homopolymerizable although this propensity was not previously recognized (lysine, arginine, and histidine). Most recently one amino acid, cystine, has been found to be thermally convertible to many amino acids which are simultaneously copolymerizable. This conversion occurs at 100° C in the presence of hypophosphite and polyphosphoric acid.

The thermal copolymerizability of the cystine conversion products is explainable on the basis that much aspartic acid is formed and the copolymerization occurs with the aspartic acid. Another possible explanation is that, under the conditions employed, the copolymerization occurs directly with cystine. Of most interest is the suggestion that cystine might function as a kind of mother substance for many amino acids.

Structure of Proteinoids (Harada, Genaux, Fukushima, Fox)

A method for quantitative determination of imide linkages has been developed by ammonolysis and estimation of amide groups. The results of application of this method to the imide forms of polyaspartic acid, 2:2:1-proteinoid, and 2:2:3-proteinoid reveal that a major fraction of the aspartic acid residues are in the imide form, confirming the earlier less quantitative inference from infrared studies.

A typical proteinoid molecule is now recognized as having a mean molecular weight of 5,000 - 10,000, some proportion of each of the eighteen amino acids common to protein, one N-terminus per molecule on average, and 2-4 C-termini

per molecule on average. Accordingly, the molecules are branched, but not highly branched.

The proteinoids have been found to have reducing properties. On carboxymethylation and hydrolysis, carboxymethyl-S-cysteine has been isolated on the analyzer column. The fact that, on standing, the SH gradually disappears from such polymers suggests the formation of -S-S- bonds, a feature indicated by other evidence also.

The Bradbury-Akabori hydrazinolysis method for C-terminal analysis has been adapted to the automatic amino acid analyzer. The results on proteinoid extend the earlier data and reaffirm the inference that the distribution of the amino acids in the chains is nonrandom. If the arrangements were random, the composition in any one position should be the same as in the total composition. The results in either the N-terminal or C-terminal position (each conveniently assayed) demonstrate nonrandomness (Table I).

TABLE I

Total N-Terminal and C-Terminal Compositions of Thermal 2:2:1-Proteinoid

	N-Terminal Analysis	Total Analysis	C-Terminal Analysis
Aspartic acid	6%	71%	1%
Glutamic acid	46	11	8
BN *	48	17	91

*Collective basic + neutral amino acids.

These results provide a basis for understanding in a salient context the origin of order at the molecular level. This kind of order would be self-generated rather than controlled from the outside. Such a result first brought into purview (as subsequently did self-assembling macromolecules) a fundamental evolutionary principle which conceptually spans prebiological and biological evolution.

Imide Structure and Catalytic Activity (Rohlfing, Fox)

The relatively high catalytic activity of proteinoids for the unnatural, model substrate, p-nitrophenyl acetate, has been reported earlier. This activity is destructible in a highly reproducible way by treatment in hot water or by treatment with alkali. These treatments are immediately remindful of conditions used for the denaturation of proteinaceous enzymes and this possibility has been investigated. Recently, however, Bernhard and coworkers have presented reasons for believing that the generally unstable aspartimide linkage is an essential part of the active site of esterases. The aspartimide linkage appears in large proportion in the thermal copolyamino acids of aspartic acid, including the proteinoids. These materials are thus well adapted to investigation of this structure.

Studies of the rate of loss of the imide linkage by titration, by hypochromicity, by viscosity, and by infrared spectral analysis show that the catalytic activity is a function of the imide linkage. Inasmuch as the imide-type of polyaspartic acid has no activity and simple derivatives of histidine have relatively little activity and are not inactivatable, the necessary minimal structure combines aspartimide and histidine residues in macromolecules, presumably in suitable steric relationship.

The possibility of an easily disruptable structure which depends upon a special intramacromolecular relationship other than the mere inclusion of histidine and aspartimide groups is not ruled out by the results to date. In particular, the most active proteinoids are most easily understood as containing a very special structure.

Thermal Polymerization of Mononucleotides (Schwartz, Bradley, Fox)

By modifying the polyphosphoric acid which has been earlier used to polymerize amino acids thermally, G. Schramm has claimed successful polymerization of mononucleotides. At Wakulla Springs in October, 1963, Schramm voluntarily offered reasons why chemists in America have been unable to repeat his reported syntheses. He stated that adequate details for the synthesis will be published. He uses an ethyl derivative of polyphosphoric acid, which is made with chloroform and ether, and thus is open to serious question as a reagent occurring spontaneously in the natural environment.

With the finding in this laboratory that macromolecules as complex as proteinoids could arise thermally, without polyphosphoric acid or with it, attempts to form polynucleotides began in 1958. Recently, success has been achieved with unmodified polyphosphoric acid in the polymerization of cytidylic acid. The polymers show appropriate u.v. absorption, the correct shift in u.v. under alkaline treatment, hyperchromicity to the expected degree, susceptibility to ribonuclease, and other properties of interest.

The polyphosphoric acid can be visualized itself as a thermal condensation polymer of phosphoric acid in the primitive environment.

Synthesis of "Active Site" of Enzymes (Fox, Hayakawa, Harada)

The active site of many enzymes has been shown to contain α-L-aspartyl-L-serylglycine. The synthesis of this tripeptide has been needed in order to have material which might be copolymerized into macromolecules to test for catalytic activity. Many laboratories have failed in synthesis of the tripeptide due to lability of the synthetic intermediates. This synthesis has now been accomplished in this laboratory by producing and hydrogenolyzing the intermediate α-N-carbobenzoxy aspartyl-O-benzyl-L-serylglycine dibenzyl ester. All four substituents are simultaneously removable by hydrogenolysis.

The tripeptide itself has no catalytic activity in the hydrolysis of p-nitrophenyl acetate.

Synthesis of Leuchs Proteinoid (Fox, Hayakawa)

The possibility of a more fundamental understanding of protein nutrition emerges from the use of thermal proteinoids. Emil Abderhalden, L. B. Mendel, etc. sought proteins deficient in individual amino acids for this purpose. Such proteins were unavailable. W. C. Rose used mixtures of pure crystalline amino acids for such studies. As Rose has indicated, the information he obtained is imperfect due to caloric imbalance. Also, proteins release amino acids gradually by digestion during normal nutrition. The closest approach to normal proteins is the proteinoids. These can be used because the proteinoids are utilized nutritionally by rats (Knappen and Krampitz, Nature, 197, 289, 1963). A more suitable "synthetic protein" for nutrition would result from Leuchs anhydrides which retain optical activity much better than do heated free amino acids. Such proteinoids have now been synthesized by an intricate process. Figs. 1a and 1b show two Leuchs proteinoids, one lacking histidine.

Properties of Microspheres (Fox, Yuyama, Fukushima, McCauley)

Some of the properties of microspheres are under continuous study. Accordingly, some of the properties reported here have been treated in the previous annual report in a less complete way. The accumulated information on these properties which are reportable are, for this reason, mostly summarized here.

Origin and Composition

As either cellular models or precellular models, the microspheres from proteinoid are unique in that they arise from polymers which in turn are produced from simpler molecules. This is in contrast to many models studied earlier. Oparin's coacervate droplets are, for instance, produced from such biological polymers as gelatin.

The composition is, furthermore, much like that of the structural protein of cells. The microspheres, for instance, contain peptide-bonded tryptophan, cystine, and tyrosine; these amino acids are absent or almost absent from gelatin. The Gram stain can be applied to these units because the composition

Fig. 1a.

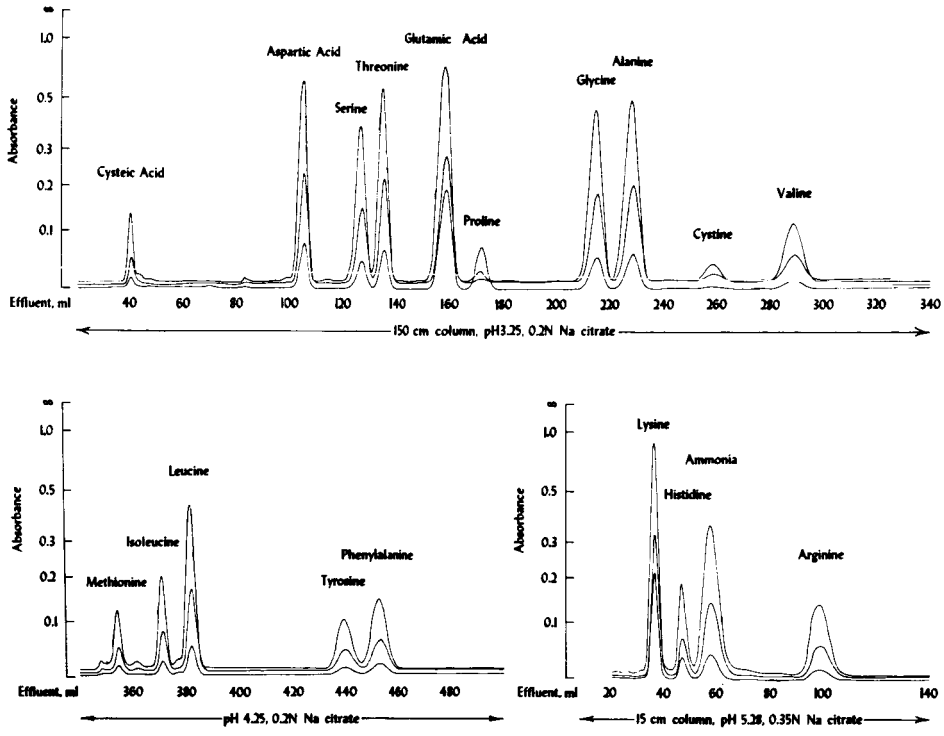


Fig. 1b.

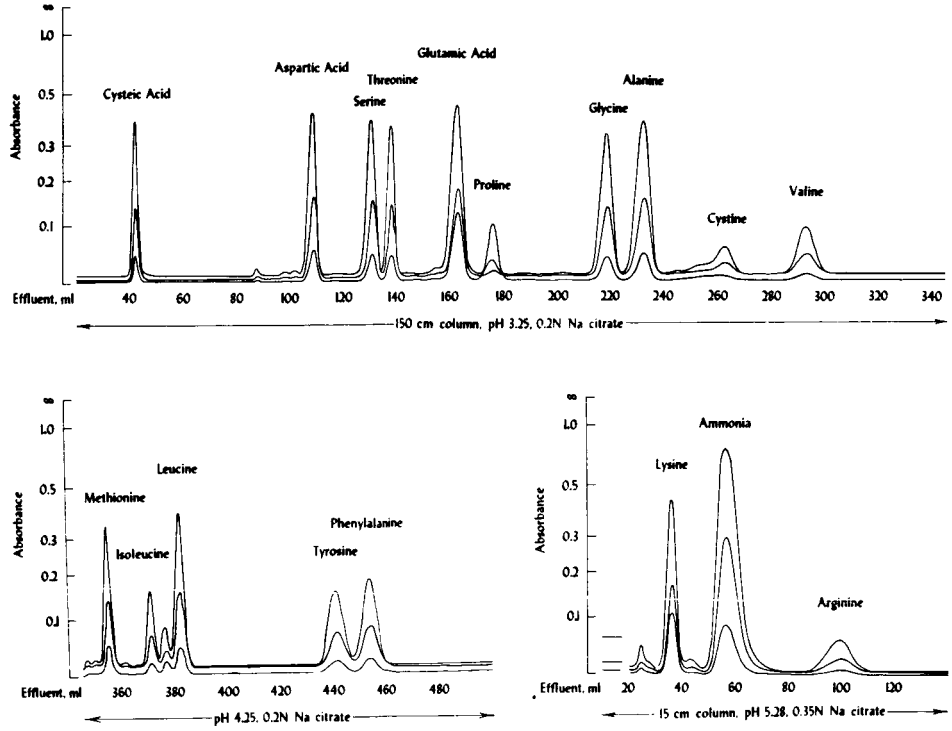


Fig. 1a. Leuchs proteinoids, all common amino acids.

Fig. 1b. Leuchs proteinoids, lacking histidine.

of the microspheres is much like that of protein in true cells. (The original suggestion that Gram-stainability of bacteria is possible because of bacterial protein was made by Stearn and Stearn in 1924 (J. Bacteriol. 9, 463). Appropriate variation in composition controls the sign of the Gram stain. Basic proteinoid in the proper proportion (35% of the total proteinoid) yields a Gram-positive stain; lower proportions give a Gram-negative stain.

Stability

The microspheres are producible in a range of stability which is comparable to that of true cells. They can be centrifuged at 3,000 rpm without losing their integrity. As shown originally by Dr. Richard S. Young, the microspheres can be sectioned, permitting electron microscopy. They can stand indefinitely with little or no visible change.

Physical Appearance and Properties

The microspheres are numerous, 10 mg. of proteinoid yielding typically 10^7 - 10^8 microparticles. Although uniformity, in some experiments, is of a very high degree, some individuality is apparent. When this variability is superposed upon the size of one natural experiment and upon the concept of an almost limitless number of highly varied natural experiments, the laboratory demonstration emphasizes the huge number of opportunities afforded for the emergence of a living unit from such spontaneous experiments in nature.

The units are microscopic. Although the exact size can be forced by the conditions used, microspheres in the range of size of 0.5 to 3.0 microns in diameter are most easily produced. The fact that the size and shape are those of the "primitive" coccoid bacteria (Kluyver and Van Niel, Lamanna and Mallette, Basic Bacteriology, 1959; Lipmann, Origins of Prebiological Systems, 1964) is provocative.

The uniformity of size which can be obtained permits an osmometric type of experiment (see below). In this property the microspheres also resemble a bacterial population much more than they do oil droplets or coacervate droplets. This property is pertinent to extraterrestrial life detection.

Chemical Properties

The stainability and the differentiation between Gram-positive and Gram-negative have been treated in a previous section.

The solubility in dilute alkali which characterizes Gram-negative bacteria is found to apply to Gram-negative microspheres, while the relative insolubility in dilute alkali of the Gram-positive type pertains also both to the bacteria and to the microspheres.

With the finding of a reproducible procedure for incorporating zinc into microspheres the door is open to many studies of chemical activity. Only the first of these is the ability to split ATP. In particular, a survey of which ATP-dependent reactions can be coupled with the microspheres of this type invites experiments.

Other Static Structural Characteristics of Microparticles from Proteinoid

Some of the microspheres appear to be "budding" as in yeasts. At least one instance of enlargement of "bud" has been observed in a time-lapse sequence but this requires several kindred observations before a definite assertion is justifiable.

Other microparticles are filamentous. The filamentous type is an association of very small particles of uniform dimension. This population of microspheres of a second size tends to appear in microscopical fields in which a divisional type of phenomenon is observed in much larger microspheres (Fig. 3a I[a]).

The electron micrographs (Fig. 3b I) reveal a degree of structure which is comparable to that depicted for Bacillus cereus by Murray. Simulation of granular cytoplasm is seen.

The kind of feature observed when the pH of the suspension of microspheres is raised is the double layer (Figs. 4a and 4b I). This feature was not sought. Double layers have also frequently, however, been observed in the optical microscope but the evidence from electron micrography is more convincing.

[a] I indicates First Annual Report.

No phospholipid is present in these double layers. The hydrophobicity postulated as necessary could be provided by the hydrocarbon sidechains of residues of neutral amino acids. Recently Green and Fleischer have found the layered structure of mitochondria to be undisturbed when these are made lipid-free by extraction with aqueous acetone. They ascribe the form to "structural protein", an emphasis consistent with the inferences from the thermally prepared microspheres.

Other Dynamic Structural Characteristics of Microspheres from Thermal Proteinoid

When microspheres are transferred to solutions hypotonic or hypertonic to those in which they are made, swelling or shrinking occurs. This kind of behavior is manifest at concentrations much higher than those which induce comparable changes in biocells. This property is to be studied further in microspheres in which biolipids or thermosynthetic lipids are present.

The microspheres show selective retention of polysaccharides as contrasted to monosaccharides. The experiments dealing with this property were carried out by producing microspheres in 2.0% solutions of glucose, fructose, glycogen, or starch and washing four times with water. Although this result suggests the operation of permeability factors, the explanation of adsorption has not been ruled out.

A more compelling type of evidence of permselectivity is the observation that the polymer in the interior diffuses out through the boundary while the boundary material remains essentially intact. This phenomenon has been observed in comparison of photographs through the light microscope, in electron microscopy (Figs. 4a and 4b I[a]) and in time-lapse studies (Fig. 6 I).

The kind of result suggesting that particles have cleaved with rise in pH is shown in Fig. 5 I. The question of whether such units are actually divided bacteria has been raised; this possibility has been rigorously excluded. The

[a]I indicates First Annual Report.

question of whether the units observed are the result of fusion or fission is answered in Fig. 6 I[a]. For experiments of this type, the observation has been always one of cleavage.

New Perspectives

Several new perspectives could be constructed this year because of the maturation of interpretations from new configurations of data.

Thermal Theory

With the finding by Dr. Harada that "primordial" gases are convertible at temperatures of 950 - 1050° to at least fourteen of the common amino acids, the thermal theory is enlarged. Increased reason to believe that the primitive earth produced important organic substances in the same way that an organic chemist would, i.e. by heating, is at hand.

The total results suggest a sequence of prebiological events from primordial gases to ATP-splitting precellular forms by thermal transformations, with liquid water entering at the appropriate moment:

NH_3, H_2O, CH_4 $\xrightarrow{950\text{-}1050^{\circ}}$ almost all amino acids $\longrightarrow$ preprotein $\xrightarrow{Zn(OH)_2}$ ATP-active protocells

This sequence is inferred only from coordinated experiments. It has been shown to occur within less than twenty four hours. The fact that the formed units emerge in water and are soon covered by water indicates how the organic material of which they are made would be protected from the destructive effects of continued heat and of other forms of radiation. Experts who have worried about such destruction have typically assumed exposure to destructive effects over tens of thousands of years.

When one superposes billions of years of experiments upon the evidence which now indicates that natural thermal experiments not only could, but must,

[a] I indicates First Annual Report.

have occurred innumerable times during any outpouring of terrestrial basalt, and also recognizes the astronomical number of slightly varied individual microparticles in each of these experiments, he is led to a new outlook. This outlook emphasizes the adequacy of a great number of opportunities for one or more of these experiments to incorporate self-replicating properties into the unit. The likelihood that other natural experiments will yield the total properties necessary is conceptually enhanced by these recognitions.

Resolution of the Paradox of Primordial Protein

The dilemma of the first protein molecule has been very clearly stated by Blum in his Time's Arrow and Evolution, second edition, Princeton, University Press, 1955, p. 170, as follows:

"---. The origin of life represents a transition from the nonliving to the living, which I have great difficulty in imagining as a sharp one. I do not see, for example, how proteins could have leapt suddenly into being. ---. The riddle seems to be: How, when no life existed, did substances come into being which today are absolutely essential to living systems yet which can only be formed by those systems? (Italics Blum's). It seems begging the question to suggest that the first protein molecules were formed by some more primitive 'nonprotein living system', for it still remains to define and account for the origin of that system."

The thermal experiments have demonstrated how a kind of primitive protein could have leapt suddenly into being from a nonliving system, i.e., a prebiological geological locale. These thermal protein-like polymers are difficultly, if at all, distinguishable from bioprotein. In particular, the properties which they have include a morphogenicity which is expressed as a tendency readily to form cell-like units.

Evolution of Protein Synthesis

Regardless of how similar the thermal proteinoids are to contemporary cellular protein, cells make their protein by another mechanism. Recent experiments suggest how a prebiological synthesis of protein might have

modulated to a biological synthesis. The units which form from thermal proteinoid can incorporate catalytic zinc in such a manner that the zinc-containing particles split ATP. This experimental demonstration indicates that further natural experiments might have led into ATP-dependent polymerizations, as of amino acids, in association with such formed units.

Priority in the Origin of Protein or of Nucleic Acid

Some authors in the area have been concerned with whether nucleic acid originated before protein or vice versa. The work in this laboratory admits of either possibility or of the third possibility that they originated simultaneously. Another perspective entertains the possibility that the fundamental property of replication is a property of the whole system in which the coding ability of nucleic acids and the catalytic and permselectivity properties of proteins is each crucial but so also is their coordinated interaction.

Insofar as it may be possible to answer these questions through experimental models, each of the mechanisms mentioned is being investigated: a) an initial (thermal) abiotic synthesis of polynucleotides, b) an initial abiotic synthesis of protein or metal-protein which may function as a mononucleotide polymerase and c) an integrated system. The argument revolves not around which material is more necessary for replication but about which came first.

The case for an initial synthesis of genic material has been presented by Müller (Persp. Biol. and Med., 5, 1, 1961). The possibility of an initial self-replicating protein has been suggested by Lederberg (Science in Space, p. 415, 1961). Pattee has shown in a theoretical way that primitive protein must contain some information (Biophys. J., 1, 683, 1961), and this laboratory has demonstrated that the thermal interaction of amino acids (each being of unique shape and distribution of charge) results in some measure of self-ordering (Fox and Harada, Science, 128, 1214, 1958; Fox, Amer. Inst. Biol. Sci., Jan. 1959).

Simulation of Formed Elements in Meteorites (Fox Yuyama)

The various microparticles seen in experiments in this laboratory mimic each of those, except one, which have been published by Claus, Nagy, etc. as

formed elements from meteorites. This exception has been shown by Gridley staining by Dr. Edward Anders, to be a ragweed pollen grain.

These and other results permit three inferences: 1) The formed elements are perhaps prebionta rather than protobionta. 2) Since no genes are present in these and related experiments, the self-organizing propensities of polyamino acids are sufficient to explain morphologies (likewise in cells). 3) The experiments done in the laboratory are close to natural experiments, especially inasmuch as laboratory conditions prevail in the meteorites (Mason, Meteorites). Comparisons are pictured in Figs. 2a and 2b, 3a and 3b, and 4a and 4b.

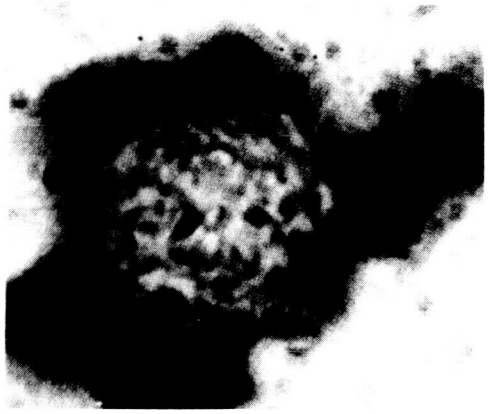


Fig. 2a.

Fig. 2b.

Fig. 2a. "Life-like" microparticle from Orgueil meteorite (Claus, G., Nagy, B., and Europa, D. L., Ann. N. Y. Acad. Sci., 108, 580-605, 1963).

Fig. 2b. Microparticle of thermal proteinoid from this laboratory.

Fig. 3a.

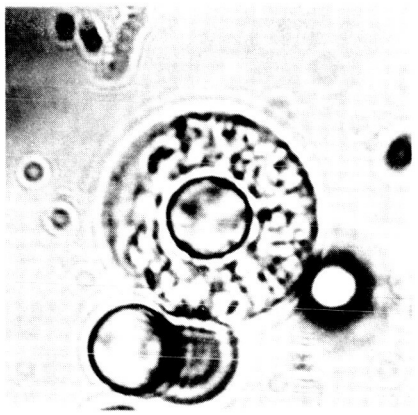


Fig. 3b.

Fig. 3a. As in 2a.

Fig. 3b. As in 2b.

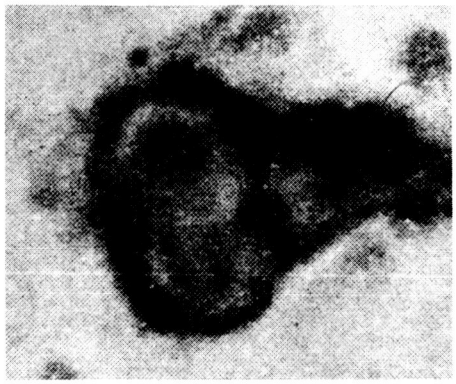


Fig. 4a.

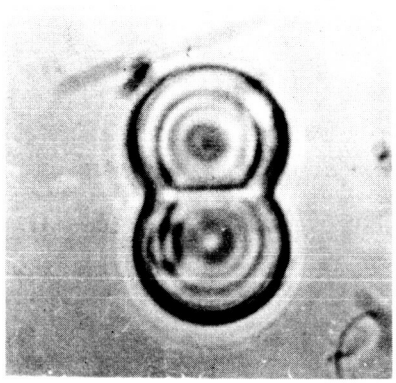


Fig. 4b.

Fig. 4a. "Life-like" form undergoing "cell-division", from Orgueil meteorite. (Claus, G., and Nagy, B., Nature, 192, 504-506, 1961).

Fig. 4b. As in 2b, pH has been raised moderately.

All microparticles are roughly in same range of size.

Report on Reports of Krishna Bahadur

Dr. Krishna Bahadur was appointed as research associate in this institute in November 1962. After a few months of a renewable annual appointment, Dr. Bahadur left at a time when he claimed to have produced microspheres in a manner that caused them to grow, divide, and metabolize. Attempts to repeat his experiments in this laboratory failed. Subsequently Bahadur sent to this laboratory a manuscript describing the work recorded in his notebooks. This manuscript had the name of S. W. Fox on it. S. W. Fox demanded that his name be removed. Bahadur's purported results and claims were next published in the Allahabad Leader. A copy of a letter by S. W. Fox to that newspaper follows this report. More recently a journal paper by Bahadur, without S. W. Fox as coauthor, has appeared in Vignana Parishad Anusandhan Patrika.

In what Bahadur claims to have seen, he failed to use adequate quantitative control. For instance, he claimed fermentation by microspheres in nutrient suspension in sterile flasks, having seen frothing on agitation. He did not, however, shake the control flask in the same fashion. When this was done in the repetition, frothing resulted in the control. The experimental directions of Bahadur were exactly followed also at the Ames Research Center, Mountain View, California by Dr. Richard S. Young and (Mrs.) Barbara McCaw. Increases in number up to six-fold were observed in periods of about two weeks with shaking according to directions but not without shaking. No increase in size was observed in any case. Decrease in size was observed in accord with the observations in the repetition by (Mrs.) Elizabeth Wiggert in Tallahassee. No increase in total mass was observed in any of the experiments.

The main conclusion from these attempts to repeat Bahadur's claimed results must be that the vigorous agitation specified in the directions is breaking the microparticles into smaller particles, comparable to Oparin's observation with coacervate droplets.

The degree of overinterpretation of results by a visiting investigator claiming units which grow, divide, and metabolize can be more accurately judged when one recognizes that Dr. Bahadur found in the laboratory he visited

microspheres which are enlarged under some conditions (Fox, Harada, and Kendrick, 1959) which simulate septate division (First Annual Report, Institute for Space Biosciences; Fox and Yuyama, Ann. N. Y. Acad. Sci., 108, 487, 1963; ibid., Comp. Biochem. Physiol., 11, 317, 1964), and which can split ATP (this report, and Fox (ed.), The Origins of Prebiological Systems, 1964).

The Leader, Allahabad

23 June 1963

p. 3

Dr. Krishna Bahadur's experiments

Sir,—Cuttings of articles in your paper describing reports by Dr. Krishna Bahadur have been sent to me. My name has been used in connection with one of those articles without my authorization.

Having published in the 1950s some eye-catching scientific papers on the origin of amino acids, Dr. Bahadur was appointed as research associate in this laboratory on an annual basis in November, 1962. He left here in April, 1963 at a time in which others were trying to reproduce the experimental results he claimed. We have not been able to repeat the principal results recorded here by Dr. Bahadur even though our laboratory has accumulated many man-years of experience with non-living particles produced from thermal polyamino acids. In checking on Dr. Bahadur's results, we followed our regular procedures in retesting what may be new advances. Many of our findings have already been published in the literature of science, including pictures like those in your paper, after those findings survived rigorous experimental repetitions.

I am writing to you to disclaim any responsibility for any of the special results or interpretations of experiments conducted here by Dr. Krishna Bahadur as reported in your newspaper.

S. W. FOX

The Florida State University, Tallahassee, June 13, 1963.

The Outlook for Synthetic Proteinaceous Nutrition

Many difficult problems can be visualized along the road to the control of synthetic nutrition in such a way as to support a cyclical chemical system of nutrition by conversion of human wastes. The developments, recorded in this report and others, of pansynthesis of amino acids and panpolymerization of amino acids under relatively simple operational conditions permit devoting more serious attention to this potential chemical development. A gross outline can be visualized as follows:

$$\text{Human wastes} \xrightarrow[\text{temp.}]{\text{High}} \begin{matrix} CH_4,\ CO_2 \\ N_2,\ NH_3 \\ H_2O \end{matrix} \xrightarrow[\text{temp.}]{\text{High}} \text{amino acids} \xrightarrow[\text{temp.}]{\text{Lower}}$$

$$\text{proteinoids} \xrightarrow{\text{nutrition}} \text{human wastes} \longrightarrow \text{, etc.}$$

The possibility of this kind of cycle is now not without experimental encouragement.

An economical synthesis of food components is needed for sustaining one or more investigators on the surface of the Moon for at least a year (ex post facto reference to address by Dr. Edward Teller at Tampa conference on space nutriton, April, 1964).

Theoretical Studies of the Atmosphere of Venus

Vertical Structure Above the Cloud Surface of Venus (Hess, Joern)

Capt. Joern is continuing numerical studeis of this problem using the high-speed computer at Offut Air Force Base, Omaha.

Vertical Structure Below the Cloud Surface of Venus (Hess)

It has been ascertained that the model being used is subject not only to gravitational instability and to linear computational instability, but also to a kind of non-linear computational instability. Methods of coping with the first two kinds of instability have been devised but work is continuing on the non-linear instability. At least two methods of dealing with this phenomenon are known. The problem is to make certain that physical reality is not vitiated by the means adopted and to avoid use of exorbitant amounts of computer time.

Physical Models of the Jupiter Atmosphere (Hess, Hadlock)

The basic rotating apparatus has been completed by the machine shop and a period of adjustment and test has been gone through. Certain mechanical and photographic problems have been encountered but we are confident of being able to solve these. A series of survey experiments is about to begin, the purpose of which is to evaluate the types of flow patterns produced over a suitable range of the parameters under our control. We already have an indication that the geometrical distribution of heat sources and sinks we have adopted as appropriate to a Jovian model produces flow patterns of a type never before seen in such experients.

Instrumentation for Measurement of Water Vapor in the Atmosphere of Mars (Hess, Cheng)

Apparatus for testing our second conception of a frost-point hygrometer is almost complete. Evaluation of this equipment will begin shortly.

Theoretical Interpretation of New Data on the Martian Atmosphere (Hess)

Certain new information, both published and unpublished, on the atmosphere of Mars have recently become available. These deal with the identification of

water vapor and a revision upward of the amount of carbon dioxide. The latter implies a revision downard of the estimated surface pressure. This problem has been examined under the assumption that only three constituents contribute significantly to the total pressure: nitrogen, argon, and carbon dioxide. The results indicate that, even for a very high proportion of carbon dioxide, the surface pressure exceeds 35 mb. Thus any downward revision of the pressure from the currently accepted 85 mb would not be so extreme as the 10 to 20 mb suggested privately by investigators at the Jet Propulsion Laboratory.

Physiology of Reproduction (Metz and Associates)

Research and instruction in the general area of the physiology of reproduction proceeded on several fronts. These may be categorized as follows: morphology of gametes and of fertilization; immunochemical and related studies on reproductive mechanisms; biochemical and physiological studies on vertebrate reproduction and fertilization studies on microorganisms.

Morphology of Gametes and Fertilization

Sea urchins provide the favorite material for basic studies on fertilization. Gamete morphology and the events of fertilization have been studied by others rather thoroughly at the light microscope level and the gametes have been examined individually with the electron microscope. However, the more thorough electron microscope studies of sperm-egg interaction at fertilization have been made on other material. Therefore, it seemed desirable to undertake an exhaustive study of sea urchin sperm-egg interaction with the electron microscope. The emphasis was placed on interaction of sperm and oocytes because of the relative ease of study. Oocytes, as opposed to mature eggs, react polyspermically on insemination. Numerous sperms attach to and penetrate the oocytes with the formation of blebs (exaggerated fertilization cones) at the site of sperm entry. Such inseminated oocytes do not undergo development. Although they do not substitute for monospermically fertilized, mature eggs, they do provide a model that does not involve the artifically induced polyspermy frequently employed with mature eggs to improve the low probability of obtaining sections through the critical sperm entry region. In addition, the inseminated oocytes are of intrinsic interest themselves.

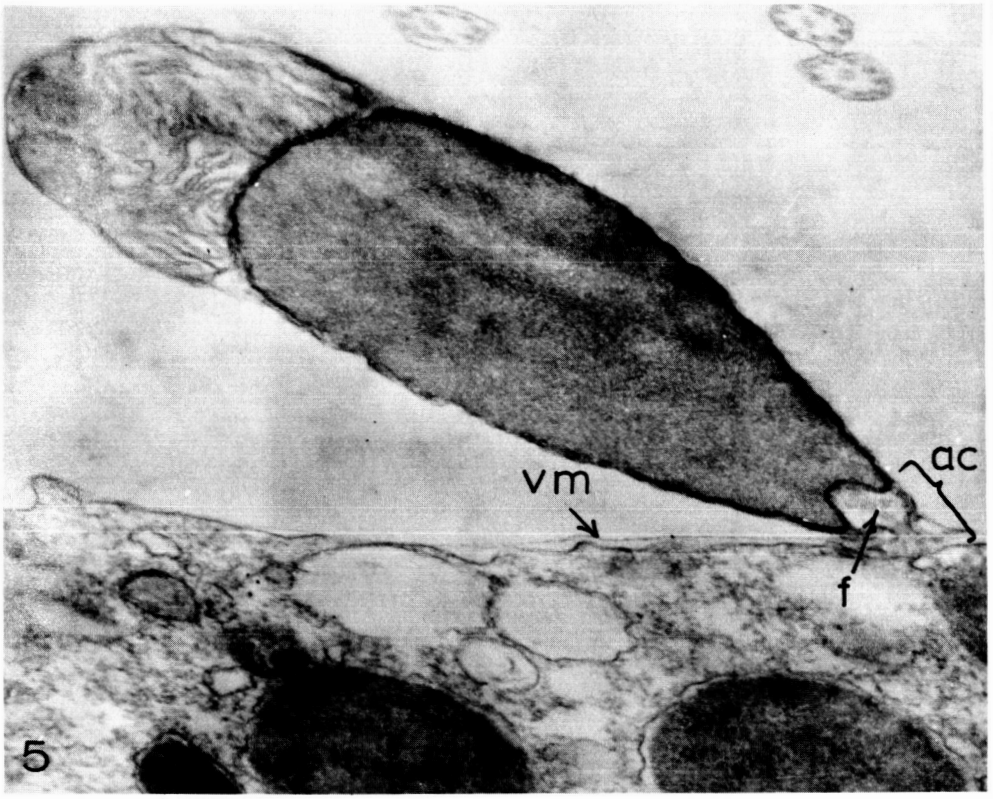
A number of details of sea urchin sperm structure were clarified. In particular, strong evidence for proximal as well as distal centrioles were found in the midpiece region. In addition, an eccentrically located centriole-like, third structure was observed. The origin and function of this "third centriole" is obscure. An "apical vesicle" comparable to that of Hydroides sperm was found in the sea urchin sperm acrosome region. This structure

is probably involved in the initial stage of the acrosomal reaction. Additional details of sperm acrosomal morphology include a fibrillar structure in the main part of the discharged acrosome and acrosome granule papillae, comparable to those in Hydroides in the unreacted acrosome.

The thin vitelline membrane of mature eggs could not be demonstrated in oocytes. However, the delicacy of this structure is such that its absence in oocytes could not be conclusively demonstrated. Consequently, the relation of this structure, if present, to the plasma membrane and microvilli could not be demonstrated. Observations of mature eggs favor the interpretation that the vitelline membrane is continuous over the entire surface of the egg.

Membrane fusion of sperm and mature eggs and of sperm and oocytes was studied in thin sectioned material. Fusion began at the anterior, thin-walled tip of the everted tubule of the reacted acrosome (Figs. 5-8). Thus, fusion involved a "new" sperm surface. Fusion of sperm and egg membranes resulted in direct contact between substances inside the acrosomal tubule and the egg cytoplasm.

Sperm entry into oocytes closely paralleled the analogous phenomenon in Hydroides (Colwin and Colwin). Early stages of entry were characterized by progressive engulfment of the sperm nucleus and then the midpiece by the hyaloplasm of the rapidly forming fertilization cone. Engulfment does not resemble pinocytosis since the gamete membranes are fused rather than separate and the former sperm plasma membrane is incorporated into the expanding surface of the fertilization cone. Two distinct processes are occurring simultaneously, namely, expansion of the fertilization cone and incorporation of the former sperm cell membrane into the fertilization cone. If the rate of the first event exceeds the second, as evidenced in engulfment of the sperm flagellum, the process may more closely resemble pinocytosis. In other words, fertilization cone hyaloplasm may advance so rapidly at the posterior region of the sperm that portions of the fused, sperm-egg membrane could be engulfed as vesicles. With the possible exception of such vesicle formation no evidence was found for a pinocytotic type of sperm engulfment as proposed by Tyler. It appears then



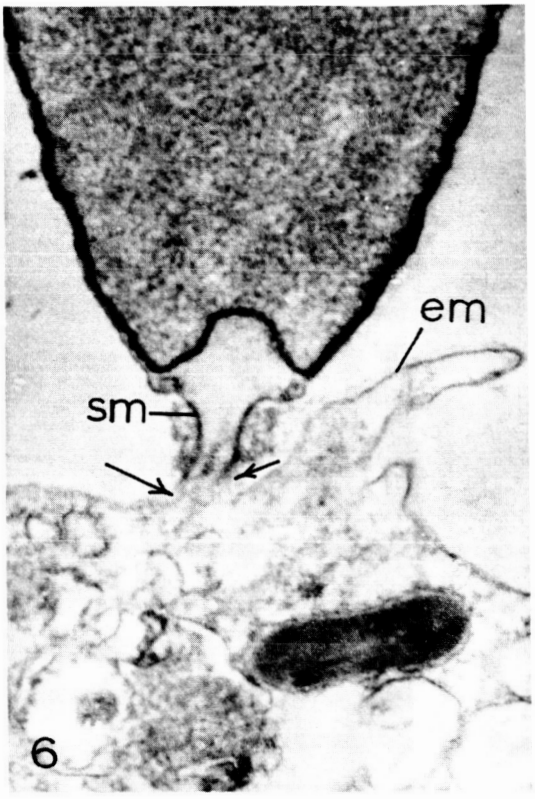
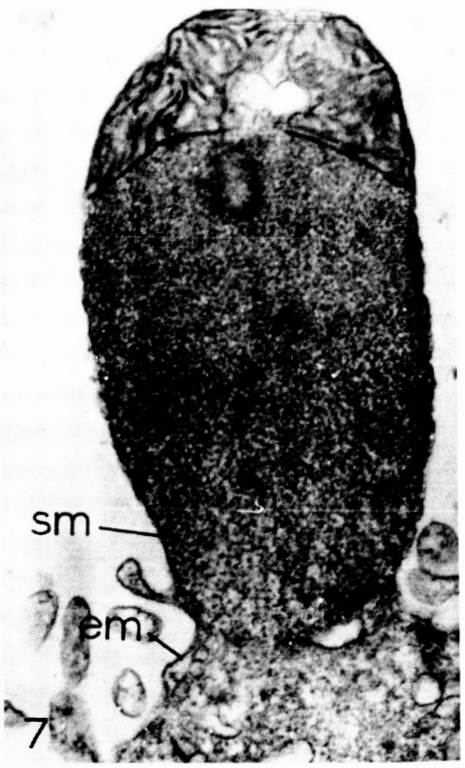


Fig. 5

Fig. 6

Fig. 5. Attachment of sperm and mature egg 30 seconds after insemination. The thin (25 Å) vitelline membrane (vm) has been penetrated by the sperm acrosome (ac). Fibrils within the acrosome (f) contact the cortex of the egg. Arbacia punctulata. x 37,500.

Fig. 6. Fusion of the membrane of the reacted sperm acrosome (sm) with the plasma membrane of a mature egg (em). The regions of fusions are indicated by arrows. Arbacia punctulata. x 57,600.



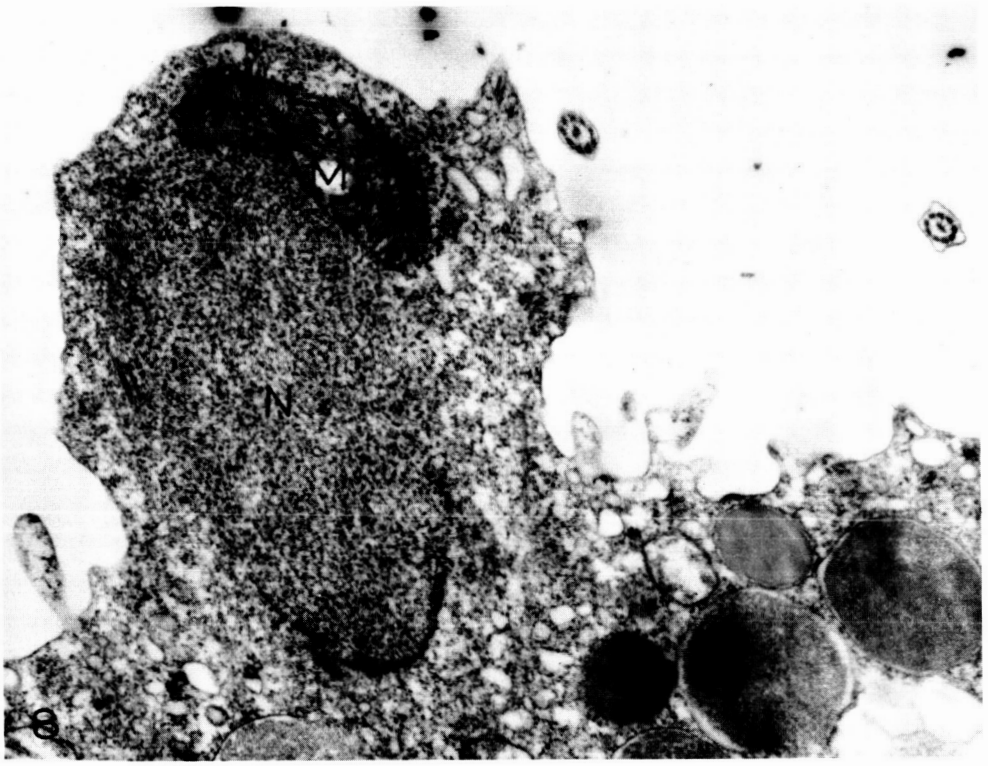


Fig. 7

Fig. 8

Fig. 7. Early stage of incorporation of a spermatozoön into an oöcyte. The plasma membrane of the sperm (sm) is continuous with the plasma membrane of the oöcyte (em). Arbacia punctulata. x 31,000.

Fig. 8. A spermatozoön incorporated within a ground cytoplasm protuberance of the oöcyte (fertilization cone). The sperm nucleus (N) and middle-piece (M) are devoid of a cell-membrane. Arbacia punctulata. x 23,000.

that sperm-egg membrane fusion is the means for engulfment of the sperm contents into the egg of the sea urchin.

Shortly after initial fusion of sperm and egg membranes it was difficult to distinguish the sperm nuclear membrane except at the nuclear apex and the region of the proximal centriole. Apparently the swelling of sperm nuclear material immediately after membrane fusion is accompanied by a change in characteristics of most of the nuclear membrane. Migration of sperm (nucleus plus midpiece structures) from the hyaloplasm of the fertilization cone into the organelle zone of cytoplasm was accomplished without any evidence of fibrillar structures being present except sperm tails. Aster formation had not begun. No evidence for aster formation or further development of the sperm nucleus was observed in these inseminated oocytes during the period examined (8-10 minutes following insemination). The exaggerated fertilization cone region where sperm entry occurs in oocytes was found to consist of three zones as reported by Runnstrom. The mechanism of this phase separation in oocytes is unknown. This study constitutes the Ph. D. thesis for Dr. Luther Franklin.

Phase and electron microscope studies on crustacean gametes and fertilization have recently been initiated by Mr. George Brown. Sperms of the blue crab and related species have a complex acrosome. Attached to the acrosome is a sac-like structure with many extensions that form "filaments or pseudopods". Cytochemical tests have shown that the sac-like structure and the pseudopods contain nuclear material whereas the acrosomal complex does not. Insult with hypotonic salt solution results in a change in the acrosome comparable to the acrosomal reaction of echinoderms, molluscs, and annelids. The complex acrosome everts yielding a central shaft and accessory material some of which appears to be jelly-like. It is possible that some of this material corresponds to the acrosomal granule of conventional sperms and contains egg membrane lysin material. No suggestion of a conventional flagellum was found.

A few thin sections have been obtained showing attachment of sperm to the egg chorion and, in addition, stages in the penetration of the chorion by the sperm. All penetrating sperms have undergone acrosomal reactions and the acrosome is the region first involved in the sperm-egg interaction. This investigation has only recently been initiated. It is anticipated that additional, interesting results will be obtained in this rather unusual material.

A series of morphological studies were carried out on vertebrate material by Dr. C. R. Austin. These include investigations on sperms of unusual structure in several forms. Notable among these were observations on sperms of certain urodele amphibians which have two flagellae, one on each margin of the undulating membrane of the sperm tail and the double flagellum structure of toadfish sperms. Finally, a series of observations were made on gametogenesis and syngamy in the primitive dicyemid mesozoan parasites of the octopus kidney.

Immunochemical Studies on Reproductive Mechanisms

"Fertilization" Antigen of Sea Urchin Sperm

Sea urchin (Arbacia) sperms are composed of a number of antigens. These include at least three surface antigens (demonstrated by cross-absorption--agglutination reactions with heterologous cross reacting species), at least four soluble antigens of which two are confined to the sperm interior and two are probably located at the sperm surface, at least one insoluble sperm surface antigen (not obtained in sperm extract) and at least three "regional" sperm surface antigens (demonstrable by the use of antiserum absorbed with sperm fragments). The sperm surface antigens demonstrated by the several techniques may not all be unique. However, there are a minimum of three sperm surface antigens.

AntiArbacia sperm antibody, following conversion to the univalent, non-agglutinating form by digestion with papain, strongly inhibits the fertilizing capacity of Arbacia sperm. Therefore, it appears that at least one of the sperm antigens is involved in fertilization. The antigen or antigens involved are probably surface antigens since it appears that antibody does not penetrate to the sperm

interior.

Of the three surface antigens (or families of antigens) demonstrable by cross absorption of anti Arbacia sperm antibody with heterologous sperm, two are not involved in fertilization. Thus, sperms of Echinarachnius agglutinate when treated with antibody prepared against Arbacia sperm. Absorption with Echinarachnius sperm fails to reduce the fertilization inhibiting action of anti Arbacia sperm serum when tested on Arbacia. Furthermore, as shown by D. E. Williams, the antibody absorbed to Echinarachnius sperm can be dissociated from the absorbing sperm at high pH. Such antibody fails to reduce the fertilizing capacity of Arbacia sperm. It is clear from these experiments that the Arbacia sperm antigen shared with Echinarachnius sperm is not involved in an essential role in Arbacia fertilization.

Absorption of anti Arbacia sperm serum with Lytechinus sperm yields a serum which still agglutinates Arbacia sperm. However, this Lytechinus sperm abosrbed serum fails to inhibit the fertilizing capacity of Arbacia sperm. Therefore, the Arbacia sperm specific antigen is not involved in fertilization.

Since Lytechinus sperm absorbs the fertilization inhibiting antibody from anti Arbacia sperm serum, it follows that an Arbacia sperm antigen shared with Lytechinus sperm is the antigen(s) essential to the fertilizing capacity of Arbacia sperm. This "fertilization" antigen is present in Arbacia sperm extracts since such extracts also neutralize the fertilization inhibiting action of the antibody. The antigen(s) in question may be associated with one of the four soluble antigens demonstrated by agar gel precipitin tests. However, the situation is complicated by the fact that the "fertilization" antigen in extracts is sedimented at relatively low centrifugal forces (28,000 g). It is possible that the antigen is associated with readily sedimented sperm fragments. However, such centrifugation results in a sperm extract that produces only three precipitin bands in agar gel precipitin tests.

Role of the Egg Jelly in Frog Fertilization

Studies on mechanisms of fertilization in the frog were performed by Dr. Shivers. The most significant recent finding was that body cavity eggs, which do not normally fertilize following insemination with untreated sperm, do fertilize when mixed with sperms that have previously been in contact with mature uterine eggs. It is believed that the sperm undergoes some change while in contact with jelly coat material of the uterine eggs which allows them to interact with and fertilize the body cavity eggs. This change in the sperm may be comparable to "capacitation" which has been reported to occur in the mammalian sperm in the female reproductive tract. The ultrastructure and change in the jelly and cortex of the frog eggs following fertilization were examined by electron microscopy.

Antigenic Composition of Pig Blood and Follicular Fluid

Availability of pig material (courtesy of the Rea Serum Company, Tallahassee, Florida) was exploited to perform a comparative biochemical and immunochemical study of pig blood and ovarian follicular fluid (Shivers, Metz, and Lutwak-Mann). It was found that follicular fluid contained less protein, protein-bound orcinol reactive carbohydrate, reducing sugars and sialic acid; but more acid soluble phosphate and citric acid than blood serum. Fructose and ergothioneine were not present in appreciable amounts in either follicular fluid or serum.

Immunoelectrophoretic analysis showed that both follicular fluid and serum contained at least 10 different antigenic components. Most of these were common to both but two of the serum antigens were not found in follicular fluid. The single follicular fluid antigen absent from serum appears to be fibrinogen.

Uterine washings contained at least six antigens most of which were not found in follicular fluid or blood.

Purification and Characterization of Mytilus Egg Membrane Lysin from Sperm

The sperm of the mollusc, Mytilus, release a lysin that evidently permits

passage of the sperm through the extracellular hyaline layer of the Mytilus egg. S. D. Haushka prepared active extracts by freeze thawing minced testes or sperm suspensions. Purification was achieved by fractional precipitation with $(NH_4)_2 SO_4$ to yield a preparation that gave a single major ultracentrifuge peak ($S_{20} = 3$) and a minor peak of slightly heavier material. The lysin was inactivated by heating for 1 minute at 100° C and did not diffuse through cellophane. Trypsin duplicated the lysin's effect upon the hyaline layer of eggs, but the lysin showed no proteolytic activity on serum albumen.

Antisera prepared against Mytilus sperm and eggs showed no effect on the reaction rate of the lysin. However, eggs pretreated with anti egg sera became completely resistant to the lysin. Evidently, anti egg antibodies combine with and protect the hyaline layer sites that are attached by the lysin. Further analysis of this interesting system will be made suring the summer of 1964 when material will again be available.

Chemical Studies on the Sperm Agglutinin, Fertilizin

The jelly surrounding the eggs of sea urchins and a variety of other organisms dissolves on standing in sea water. This jelly solution has a powerful and species specific sperm agglutinating action. This action suggests that the agglutinin, fertilizin, of the egg jelly solution may have an important if not essential role in fertilization. Considerable evidence is available to support this view. Chemically the fertilizins from a veriety of organisms including algae and yeasts have been found to be glycoproteins. The sea urchin fertilizins have certain unique features, notably that the molecules readily split yielding "univalent" fragments which combine with but fail to agglutinate sperm, probably because the univalent fragments contain only one combining site complementary to the sperm surface. The nature of this splitting is of interest both because of its possible role in fertilization and as a contribution to knowledge of glycoprotein structure.

A study in this direction has been undertaken by Mr. Samuel Stern.

In the ultracentrifuge a single peak was obtained with either Schlieren or u.v. optics. The sedimentation velocity of this was 10 s. The material shows strong u.v. absorption in the range 220-280 without notable peaks. One definite band and a possible second faint band were obtained in disc electrophoresis. The univalent, non agglutinating material does not react as strongly metachromatically with toluidine blue as does the native fertilizin.

Biochemical and Physiological Studies on Vertebrate Reproduction (Mann and Lutwak-Mann)

Studies on the Male

Much is known concerning the chemical composition of mammalian reproductive organs, particularly in the large domestic animals. The availability of a variety of material in the Tallahassee area made it possible for Dr. T. Mann and Dr. C. Lutwak-Mann to extend observations to animals other than the large domestic type. In the opossum several chemical substances were found in the male reproductive tract. In particular, one distinct region of the so-called prostate complex proved to be very rich in fructose, the same sugar that forms the main free carbohydrate in the semen of man, bull, and ram. In another region of the same complex one of the main constituents was ergothioneine. This is the first demonstration of ergothioneine in the prostate. It has previously been known from very few animals, mainly in the ampullary glands of Equidae and seminal vesicles of the boor and certain Insectivora. This finding provides another instance demonstrating that male accessory organs believed to be "homologous" on the basis of morphology, differ markedly in chemical characteristics.

A second animal examined was the raccoon, Prycyon lotor. The prostate of this carnivore contained a very high concentration of citric acid. This again is of comparative interest since previously, citric acid was known to occur in large concentrations in the human prostate, but not in that of carnivora, e.g. dog.

In addition to the mammals mentioned, a series of experiments were performed

on the composition and metabolism of semen in certain reptiles, notably the alligator, rattle snake and indigo snake. The metabolic behavior of spermatozoa in these reptiles was very similar to that of birds. Although reptilian semen, like avian semen, is devoid of fructose or other free glycolysable sugars, the spermatozoa of the reptiles and birds nevertheless have the ability to glycolyse added sugars such as glucose or fructose. Their glycolytic ability is, however, lower than that of mammals. This is in contrast to lower forms, e.g. echinoderms, which are incapable of glycolysing sugars.

In addition to the above vertebrates, five male octopi were examined. The male reproductive apparatus of this organism is complex in structure. Most interesting was the discovery of 5-hydroxytryptamine (serotonin) in the glandular portion of the spermatophoric sac. Serotonin is a powerful smooth muscle stimulant. In the octopus it may play a physiological role in the ejaculation of spermatophores from Needham's spermatophoric sac or a stimulation of contraction within the male or female reproductive tract. In certain mammals and fish, serotonin is known to strongly stimulate uterine contractions.

Studies on the Female

The early stages of pregnancy in the opossum were investigated. It was found that the typical endometrial enzyme, carbonic anhydrase, was practically undetectable in non-pregnant uteri of adults. However, in the proliferating and greatly thickened endometrium of pregnancy, carbonic anhydrase activity was comparable to that of pregnant rabbit, mice, and sheep endometria. In the involuting opossum uteri obtained following birth, carbonic anhydrase activity rapidly declined.

Several opossum blastocysts estimated to be of 4-5 days gestation were examined. DNA per blastocyst was roughly 1/3rd of that recorded for preimplantation rabbit blastocysts. In addition a preliminary study of embryonic fluids from 0.5 cm embryos revealed a remarkably high value for reducing sugars, namely 0.5 to 1.0%. The sugar has so far not been identified but does not appear to be fructose. The reducing sugar in the blood of the pregnant opossum was 135 to 150

mg. %.

Survival of Spermatozoa in the Female Reproductive Tract of Reptiles (Austin)

Numerous reports indicate that spermatozoa survive for months or years in the female tract of a variety of forms including snakes. Examination of rattle snake uteri obtained when males yield little or no spermatozoa and before the normal breeding season, revealed spermatozoa in nearly every case. These spermatozoa were closely associated with the glandular epithelium of the uterine wall. A large amount of this material was prepared for detailed cytological study. When this examination is completed by Dr. Austin, it should reveal if there is an intimate attachment of these long surviving spermatozoa to the cells of the uterus, and possibly provide information on the mechanism of sperm survival in the female reproductive tract.

Fertilization Studies on Microorganisms (Wiese)

Sexual mechanisms in microorganisms are of almost infinite variety. Aside from their intrinsic interest such materials may reveal aspects of the evolution of reproduction and provide useful models for studies on higher organisms. Dr. Lutz Wiese joined the Institute for Space Biosciences in September, 1963, to begin a study of sex substances and mating mechanisms in the green alga, Chlamydomonas, employing biochemical and immunochemical methods. Dr. Wiese's laboratory is now equipped, cultures of the algae have been established and experiments are beginning.

Mechanisms of Chromosome Evolution (Menzel)

Plant Tissue Culture Studies

Five callus strains derived from Haplopappus gracilus ($n = 2$) have been maintained through 20 transfers (2 strains, 30 months) or 12 transfers (3 strains, 12 months) on a simple, completely defined agar medium, and have been characterized as to chromosome number or numbers, response to indoleacetic acid and kinetin, growth rate, ability to initiate root meristems spontaneously, ability to develop visible chlorophyll and anthocyanin pigments under either continuous light of low intensity or alternating cycles of 12 hours dark and 12 hours of intense light, and morphological appearance. Each strain differs from all the others in one or more characteristics. Observation of mammalian tissue culture at Oak Ridge National Laboratory during June and July, 1963, led to many improvements in technique, and construction of a sterile transfer chamber completed separation of this project from the Neurospora laboratory and brought contamination under control so that repeatable results are now being obtained with liquid cultures. Although single-cell dispersion has not been attained, sufficient dispersion to permit rapid and nearly uniform contact of all cells with additives in the ambient medium has been achieved. Effort is presently directed toward characterizing the behavior of all five strains in liquid culture, with emphasis on enhancing the mitotic index. When strain 2 d is subjected to a suitable sequence and timing of exposure to 5-aminouracil, recovery, and colchicine, about 40% of the cells can be accumulated at mitotic metaphase. This clone does not respond to indoleacetic acid, kinetin, or 2% coconut milk added to the medium, unlike Haplopappus and carrot cultures studied by F. C. Steward and coworkers.

Evolutionary Divergence of Chromosomes

The two chromosomes of a haploid genome with $n = 2$ (such as chromosomes A and B of H. gracilus) represent the greatest degree of nonhomology theoretically attainable in a single organism. Chromosome A is twice as long as chromosome B at mitotic metaphase. Attempts are being made to separate samples of chromosomes

A and B by cell fractionation procedures in order to compare and contrast their properties.

Study of the meiotic pairing of the partially nonhomologous chromosomes in the F_1 intergeneric hybrid Lycopersicon esculentum x Solanum lycopersicoides and of preferential pairing in the corresponding allotetraploid are continuing. With the help of Dr. Kastenbaum of the Mathematics Panel, ORNL, and Dr. Cornell of the Statistics Department, FSU, mathematical models relating pairing observed at metaphase I to events at synapsis and at the time of chiasma formation are being devised and tested against actual data. Preliminary results from electron microscope studies of meiotic prophase chromosomes of the F_1 hybrid strongly suggest the existence of a "synaptinemal complex" similar to that found by Moses to be characteristic of pairs of animal chromosomes at pachytene.

Attempts to label the meiotic chromosomes of the F_1 hybrid with tritiated thymidine were unsuccessful. Although treated anthers showed no labelling of pollen mother cells in autoradiographs, the nuclei disintegrated within 48 hours after treatment, in contrast to controls treated with distilled water and with unlabelled thymidine. An experimental organism in which meiosis is technically more accessible is needed for this type of experiment. The Bryophyta as a group suggest themselves since many members also have few, rather large chromosomes, including heteromorphic sex chromosomes, and interspecific hybrids can be made in some general. A survey of locally available species of mosses is being made with the advice and help of Dr. Ruth Breen, to assess their suitability as experimental organisms.

GUEST SPEAKERS AND VISITORS

Dr. and Mrs. Thaddeus Mann

Dr. Fred Sisler

Dr. Harry Gold

Dr. Robert Resnick

Dr. Lawrence Steinrauf

Dr. G. N. Ling

Dr. and Mrs. Paul Doty

Dr. Harrison Shull

Dr. Austen Riggs

Dr. W. H. Langham

Dr. Bowen Dees

Dr. Harve Carlson

Dr. Richard S. Young

Dr. Cyril Ponnamperuma

Dr. Roy Heinmets

Dr. J. J. Cebra

Dr. Charles W. Metz

Dr. Henry Lardy

Dr. C. R. Austin

Dr. H. Beinert

Dr. Arthur Koch

Dr. Roger Gallet

Dr. Alberto Monroy

LECTURES AND PARTICIPATION IN CONFERENCES AND SYMPOSIA

S. W. Fox

Participation in conference on RNA and Brain Function at Brain Research Institute, UCLA, November 1962.

Lecture on An Integrated Model of Abiogenesis in Symposium on Space Biology, AAAS national meeting, Philadelphia 30 December 1962.

American Chemical Society lecture tour, February 1963.

Lecture on Origins of Prebiological Systems, University of Arizona, 20 March 1963.

Public lecture on Origin of Life, Los Angeles County Museum, 30 March 1963.

Conference on Education for Creativity in the Sciences, New York University, June 1963.

Lectures on Origins of Proteins and Cells at University of Hawaii, July 1963, during period of collecting in volcanic regions.

Total organization of, and presentation of papers in international conference on The Origins of Prebiological Systems and of their Molecular Matrices, Wakulla Springs, 27-30 October, 1963.

C. B. Metz

AAAS meetings, Philadelphia, December 1962.

Paper Immunochemical Studies on Fertilization in the Sea Urchin given at Seventh Southeastern Regional Developmental Biology meetings, University of Tennessee, March 1963.

Lecture on Immunochemical Studies on Fertilization in the Sea Urchin at New York University School of Medicine, Honors Group Lecture, 6 April 1963.

Society for Study of Development and Growth, University of Connecticut, June 1963.

Lecture on The Wiles of Eggs (Fertilization) at Northeastern University, NSF Summer High School Biology Program, 16 July 1963.

OTHER ACTIVITIES AND RECOGNITIONS

S. W. Fox

Appointed Brian Priestman Lecturer, University of New Brunswick.

Appointed advisor to American Museum of Natural History on origin of life.

Appointed chairman of session on proteins, national meeting of American Society of Biological Chemists, Atlantic City, April 1963.

Honored as past chairman at Memorial Banquet of Golden Jubilee of Division of Biological Chemistry, American Chemical Society, New York, 10 September 1963.

Advisor to National Aeronautics and Space Administration.

Biographed in Men of Space, Vol. 6, S. Thomas (1963) pp. 90-111, Chilton Books, Philadelphia.

C. B. Metz

Secretary, American Society of Zoologists.

Research Grant Committee, Florida Division, American Cancer Society.

Panel on Gravity, National Academy of Sciences.

NASA Environmental Biology Research Grant Panel.

Chairman, Developmental Biology Session, American Society of Zoologists meeting, Philadelphia, December 1962.

NIH Cell Biology Research Grant Panel (Host and ad hoc member), Wakulla Springs, April 1963.

Chairman, Fertilization Session, Society for Study of Growth and Development, University of Connecticut, June 1963.

Chairman, Fertilization and Gamete Physiology Research Training Program, Marine Biological Laboratory, Woods Hole, Massachusetts, June - August 1963.

PUBLICATIONS

Attribution Numbers

001 Fox, S. W. and Harada, K. 1963 Experiments related to the chemical origins of protein. In G. H. Bourne (Ed.) "Space Flight". Academic Press, 261-273.

002 Fox, S. W. 1962 (Book Review) "Life: Its nature, origin, and development" by A. I. Oparin. Science 136, 309.

003 Metz, C. B. and Shivers, C. A. 1962 Inhibition of fertilization in frog eggs by univalent fraction of rabbit antibody. Proceedings of the Society for Experimental Biology and Medicine 110, 385-387.

004 Fox, S. W. and Harada, K. 1962 A total resolution of aspartic acid copper complex by inoculation. Nature 194, 768.

005 Fox, S. W. and Yuyama, S. 1963 Abiotic production of primitive protein and formed microparticles. Annals of the New York Academy of Sciences 108, 487-494.

006 Fox, S. W. 1963 The outlook for synthetic foods. Food Technology 22, 388-392.

007 Fox, S. W. and Yuyama, S. 1963 Effects of the Gram stain on microspheres from thermal polyamino acids. Journal of Bacteriology 85, 279-283.

008 Fox, S. W, Harada, K., Woods, K. R. and Windsor, C. R. 1963 Amino acid compositions of proteinoids. Archives of Biochemistry and Biophysics 102, 439-445.

009 Fox, S. W., Hayakawa, T. and Harada, K. 1963 The synthesis of α-L-aspartyl-L-serylglycine. Bulletin of the Chemical Society of Japan 36, 1050-1051.

010 Fox, S. W. 1963 Experiments suggesting origins of amino acids and proteins. In J. Kastelic, H. H. Draper, and H. P. Broquist (Eds.) "Protein Nutrition and Metabolism". Special Publication no. 4, University of Illinois College of Agriculture, 141-154.

011 Austin, C. R. and Baker, C. L. 1964 Spermatozoon of Pseudobranchus striatus axanthus. Journal of Reproduction and Fertility 7, 123.

012 Mann, T. 1963 5-Hydroxytryptamine in the spermatophoric sac of the Octopus. Nature 199, 1066-1067.

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013 Mann, T. and Lutwak-Mann, C. 1963 Comparative biochemical aspects of animal reproduction. Bulletin de L'Academie Royale de Medecine de Belgique 3, 563-593.

Recent Books Presenting Extended Treatment of the
Thermal Theory of Origins

Anderson, P.: Is There Life on Other Worlds? Collier-Macmillan Ltd., 1963.

Biological Sciences Curriculum Study: Molecules to Man. Houghton Mifflin Co., 1963.

Jackson, F. and Moore, P.: Life in the Universe. W. W. Norton and Co., 1962.

Ross, H. H.: A Synthesis of Evolutionary Theory. Prentice-Hall, 1962.